STATEMENT

I, Norio Ohmori, registered Patent Attorney, having my business place at Fukuoka Building, 9th Floor, 8-7, Yaesu 2-Chome, Chuo-ku, Tokyo 104-0028 Japan, do hereby declare that I am conversant in the Japanese and the English language and that I am the translator of the documents attached and certify that to the best of my knowledge and belief the following is a true and accurate English translations of the specification of the Japanese Patent Application JP2003-171240 filed on June 16, 2003.

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[Title of the Invention] METHOD FOR PRODUCING HUMAN-DERIVED IMMUNOCOMPETENT CELLS

[CLAIMS]

[Claim 1] A newborn immunodeficient mammal (excluding human), into which human-derived hematopoietic precursor cells have been transplanted, and which is able to generate immunocompetent cells derived from said human and/or physiologically active substances derived from said immunocompetent cells.

[Claim 2] An immunodeficient mammal obtained as a result of the breeding of the newborn immunodeficient mammal (excluding human) according to claim 1.

[Claim 3] The mammal according to claim 1 or 2, wherein the hematopoietic precursor cells are derived from bone marrow, cord blood, or peripheral blood and the cells are at least CD34 positive.

[Claim 4] The mammal according to claim 1 or 2, wherein the immunocompetent cells are at least one selected from the group consisting of B cells, T cells, dendritic cells, NK cells, and NKT cells.

[Claim 5] The mammal according to claim 1 or 2, wherein the physiologically active substance is a cytokine and/or an immunoglobulin.

[Claim 6] The mammal according to claim 1 or 2, wherein the immunodeficient mammal is an immunodeficient mouse.

[Claim 7] A method for producing a mammal capable of generating immunocompetent cells derived from a human and/or physiologically active substances derived from said immunocompetent cells, which is characterized in that it comprises transplantation of human-derived hematopoietic precursor cells into a newborn immunodeficient mammal (excluding said human).

[Claim 8] The method according to claim 7, wherein the hematopoietic precursor cells are derived from bone marrow, cord blood, or peripheral blood and the cells are at least CD34 positive.

[Claim 9] The method according to claim 7, wherein the immunocompetent cells are at least one selected from the group consisting of B cells, T cells, dendritic cells, NK cells, and NKT cells.

[Claim 10] The method according to claim 7, wherein the physiologically active substance is a cytokine and/or an immunoglobulin.

[Claim 11] The method according to claim 7, wherein the immunodeficient mammal is an

immunodeficient mouse.

[Claim 12] A method for producing a human-derived antibody, which is characterized in that it comprises recovering immunocompetent cells from the mammal according to any one of claims 1 to 5, culturing said immunocompetent cells in the presence of an antigen or a stimulator, and collecting said human-derived antibody from the obtained culture product.

[Claim 13] The method according to claim 12, wherein the immunocompetent cells are at least one selected from the group consisting of B cells, T cells, dendritic cells, NK cells, and NKT cells.

[Claim 14] A method for producing a human-derived antibody, which is characterized in that it comprises immunizing the mammal according to any one of claims 1 to 5, with an antigen or a stimulator, and collecting said human-derived antibody from the immunized mammal.

[Claim 15] The method according to claim 14, wherein the antibody is collected from blood plasma or serum.

[DETAILED DESCRIPTION OF THE INVENTION]

[0001]

[Technical Field of the Invention]

The present invention relates to a technique used for the *in vivo* growth of various cells essential for immune response, such as human lymphoid cells or antigen-presenting cells, and the reconstruction of a human immune system.

[0002]

[Prior Art]

It is important for studies regarding human stem cells to conduct measurement *in vivo*. Thus, such studies are carried out based on xenogeneic transplantation using immunodeficient rodents or sheep fetuses. In 1988, McCune et al. have reported Scid-hu assay (Science 241: 1632-1639 (1988)). This assay constituted a first case where human cells were detected in a CB17/SCID mouse. Thereafter, as recipients into which human hematopoietic cells are to be transplanted, many types of immunodeficient mice have been used. Examples of such immunodeficient mice may include NOD/SCID, NOD/RAG-1^{null}, beige/nude/scid, and NOD/SCID/$\beta 2M^{null}$.

[0003]

However, a majority of mice used as recipients for transplantation of stem cells in xenogeneic animals are adult mice with an age of 8 to 12 weeks old. In addition, in the case of using common SCID adult mice, in order to maintain a graft for a long period of time,

administration of extrinsic cytokine is necessary. Moreover, it is difficult to allow T cells to differentiate from precursor cells.

[0004]

[Nonpatent literature 1]

McCune, J.M. et al., 1988. Science 241:1632-1639.

[0005]

[Nonpatent literature 2]

Pflumio, F. et al., 1996. Blood 88:3731-3740.

[0006]

[Nonpatent literature 3]

Shultz, L.D. et al., 2000. Journal of Immunology 164:2496-2507

[0007]

[Nonpatent literature 4]

Dao, M.A., and J.A. Nolta. 1998. International Journal of Molecular Medicine 1:257-264.

[0008]

[Nonpatent literature 5]

Kollet, O. et. al., 2000. Blood 95:3102-3105.

[0009]

[Nonpatent literature 6]

Flake, A.W. et al., 1986. Science 233:776-778

[0010]

[Nonpatent literature 7]

Ito, M. et al., 2002. Blood 100:3175-3182

[0011]

[Problems to be resolved by the invention]

It is an object of the present invention to construct a human immune system in a xenogeneic animal host, so that an immune reaction can be allowed to take place naturally or artificially, thereby generating necessary human immunocytes, immunoglobulins, cytokines, or the like.

[0012]

[Means for solving the problem]

As a result of intensive studies directed towards solving the aforementioned object, the present inventors have found that the aforementioned object can be achieved by transplanting hematopoietic precursor cells into an immunodeficient animal, thereby

completing the present invention.

[0013]

(1) The present invention relates to a newborn immunodeficient mammal (excluding human), into which human-derived hematopoietic precursor cells have been transplanted, and which is able to generate immunocompetent cells derived from the above-described human and/or physiologically active substances derived from the above-described immunocompetent cells. In addition, the present invention relates to an immunodeficient mammal obtained as a result of the breeding of the above-described newborn immunodeficient mammal (excluding human).

[0014]

Examples of the above-described hematopoietic precursor cells may include cells, which are derived from bone marrow, cord blood or peripheral blood and are at least CD34 positive cells (e.g., $CD34^+$ cells, or those exhibiting $CD3^-$, $CD4^-$, $CD8^-$ and $CD34^+$). The above-described immunocompetent cells are at least one selected from the group consisting of B cells, T cells, dendritic cells, NK cells, and NKT cells. Such immunocompetent cells can be collected from the peripheral blood of a recipient without sacrificing the recipient. When a large number of cells or physiologically active substances derived from the aforementioned immunocompetent cells (for example, an immunoglobulin, a cytokine, etc.) are purified, bone marrow, spleen, thymus, lymph node, or the like can be used as a source of cells. The above-described immunodeficient mammal is preferably an immunodeficient mouse.

(2) The present invention also relates to a method for producing a mammal capable of generating immunocompetent cells derived from a human and/or physiologically active substances derived from the above-described immunocompetent cells, which is characterized in that it comprises transplantation of human-derived hematopoietic precursor cells into a newborn immunodeficient mammal (excluding human). Examples of the above-described hematopoietic precursor cells may include cells, which are derived from bone marrow, cord blood, or peripheral blood and are at least CD34 positive cells. The above-described immunocompetent cells are at least one selected from the group consisting of B cells, T cells, dendritic cells, NK cells, and NKT cells. Examples of the above-described physiologically active substance may include a cytokine and/or an immunoglobulin. The above-described immunodeficient mammal is preferably an immunodeficient mouse.

(3) In addition, the present invention also relates to a method for producing a human-derived antibody, which is characterized in that it comprises recovering immunocompetent cells from the above-described immunodeficient mammal, culturing the

above-described immunocompetent cells in the presence of an antigen or a suitable stimulator, and collecting the above-described human-derived antibody from the obtained culture product. The above-described immunocompetent cells are at least one selected from the group consisting of B cells, T cells, dendritic cells, NK cells, and NKT cells.

(4) Moreover, the present invention also relates to a method for producing the above-described human-derived antibody, which is characterized in that it comprises immunizing the above-described immunodeficient mammal with an antigen or a stimulator, and collecting the above-described human-derived antibody from the immunized mammal. Such an antibody is collected from blood plasma or serum, for example.

[0015]

The present invention will be described in detail below.

[0016]

[Embodiments of the Invention]

The present invention has been completed for the purpose of allowing human hematopoietic lineage cells to differentiate and proliferate in the living body of a xenogeneic mammal, so as to reconstruct a human immune system therein. Specifically, the present invention is characterized in that human-derived hematopoietic precursor cells are transplanted into a newborn immunodeficient mammal (for example, an SCID mouse), and in that the human-derived cells are allowed to differentiate and proliferate in the above host.

1. Newborn immunodeficient mammal

In the present invention, animals used as recipients, into which human-derived hematopoietic precursor cells are transplanted, are immunodeficient mammals other than humans, and are a newborn baby with an age of 7 days or less, and more preferably a newborn baby with an age of 2 days or less.

[0017]

Examples of a mammal may include a mouse, a rat, a hamster and a guinea pig. Immunodeficient mice are preferable in that there have been many types of model animals and in that the strains thereof have already been established. The term "immunodeficient mouse" is used to mean a severe combined immunodeficiency disease mouse (SCID mouse) that lacks ability to produce T cells and B cells. In particular, an NOD/SCID/β2 microglobulin knockout mouse (NOD/SCID/B2M) and an NOD/SCID/common γ-chain knockout mouse, which do not have the activity of NK cells, are preferable. When such a newborn SCID mouse individual is used, human-derived immunocytes and hematopoietic cells can be

produced in the living mouse body at high efficiency. The aforementioned SCID mice are commercially available (Jackson Laboratory), and thus persons skilled in the art can easily obtain such mice.

2. Preparation and transplantation of cells

Hematopoietic precursor cells to be transplanted can be obtained from cord blood, bone marrow, peripheral blood, for example.

[0018]

Such cord blood (CB) cells can be obtained in the form of a clinical analyte (for example, an analyte that is to be wasted due to problems regarding the number of cells or family history as a result of clinical tests) from the Metro Tokyo Red Cross Cord Blood Bank (formerly The Japanese Red Cross Central Blood Centre Cord Blood Bank). Such CB cells can be also obtained by buying commercially available CB cells. In addition, born marrow cells can be obtained from the bone marrow bank. Otherwise, such born marrow cells can also be collected by bone marrow aspiration or obtained as cells to be wasted from among the thus collected cells. As peripheral blood, blood collected for using in a general blood test, or blood to be wasted from among such collected blood, can be used. In order to efficiently collect a stem cell population from such peripheral blood, it is also possible to collect it, after stem cells in bone marrow have been mobilized with G-CSF.

[0019]

Subsequently, monocytes (MNCs) are isolated from the above cells by density gradient centrifugation.

[0020]

Examples of cells used for transplantation in the present invention may include cells exhibiting at least CD34 positive ($CD34^+$). That is, such cells are hematopoietic precursor cells. $CD34^+$ cells can be obtained by incubating a sample with an anti-human CD34 microbeads.

[0021]

The aforementioned sample acting as a source of hematopoietic precursor cells contains cells that have differentiated into T cells, as well as hematopoietic precursor cells. Thus, in order to eliminate such T cells, it is also possible to allow the sample to react with an antibody reacting with a T cell marker. For example, MNCs is incubated with mouse anti-human CD3, CD4 and/or CD8 antibodies. After washing the resultant, the cells are incubated with sheep anti-mouse immunomagnetic beads, so as to recover unbound cells.

Since CD3, CD4, and CD8 are all T cell markers (surface antigens), the aforementioned treatment is carried out using antibodies reacting with such antigens, so as to eliminate T cells. The cell surface antigens of the thus obtained precursor cells are CD3 negative ($CD3^-$), CD4 negative ($CD4^-$), and CD8 negative ($CD8^-$). Thereafter, the sample, from which T cells have been eliminated, is incubated with anti-human CD34 microbeads. By this operation, hematopoietic precursor cells presenting $CD34^+$ can be obtained. Thereafter, the cells are subjected to a magnetic column such that the purity of the concentrated $CD34^+$ cells becomes 90% or more.

[0022]

The mammal of the present invention can be obtained by previously applying radiation to the entire body of a recipient animal, and then transplanting hematopoietic precursor cells, which have been adjusted to a certain amount, into the recipient animal (an NOD/SCID/B2M mouse or the like). The number of cells to be transplanted can be determined, as appropriate, depending on the type of an animal. For example, when hematopoietic cells are transplanted into an SCID mouse used as a recipient, the number of the cells to be transplanted is at least 1×10^3 per mouse. The upper limit is not particularly limited. Preferably, 1×10^3 to 1×10^7 cells can be used. It is anticipated that the use of a large number of cells enable differentiation of human cells at higher efficiency.

[0023]

Such cells are preferably transplanted intravenously. Intraperitoneal, intracardiac or intrahepatic transplantation may be employed. When cells are transplanted intravenously, such cells are injected via facial vein or caudal vein. In such a case, an injection needle with 26 to 30 gages (G) may be used (for example 29G). For example, it is preferable that 1×10^5 of CB cells ($CD3^-CD4^-CD8^-CD34^+$), from which T cells have been eliminated, be transplanted by intravenous injection into a newborn NOD/SCID/B2M mouse, the entire body of which has previously been irradiated with 100cGY.

[0024]

After transplantation of the cells, the mouse is bred while carefully maintaining an aseptic environment. The term “maintenance of an aseptic environment” is used to mean that the environment is controlled such that it does not contain pathogenic microorganisms causing infectious diseases or antigenic substances. Thus, this means that mice are bred in an aseptic room at so-called SPF (specific pathogen free) level, are fed an irradiated diet (or a low molecular weight diet), or are fed sterilized water. In the case of mice, if mice are bred in the aforementioned aseptic environment for 2 to 16 weeks, and preferably for 3 to 4 weeks, they

can be used for the recovery of immunocytes or immunization. The present invention also provides the thus bred mammals.

[0025]

A donor- (human-) derived immune system has been established in the body of the thus obtained mammal. Thus, human-derived immunocompetent cells or the like can be recovered therefrom. In the present invention, the term "immunocompetent cells" (which is also referred to as immunocytes) is used to mean cells used for establishing immune response. Examples of such cells may include antibody-generating cells and hematopoietic cells. Specific examples may include B cells, T cells, dendritic cells, NK cells, and NKT cells.

[0026]

The ratio of such human-derived cells to recipient-derived cells is between 5% and 90%, and preferably between 20% and 90% in the case of hematopoietic cells. Such ratio is between 2% and 80%, and preferably 10% and 80% in the case of antibody-generating cells.

[0027]

The aforementioned immunocompetent cells are cells derived from a human used as a donor. Various types of physiologically active substances are generated from such cells. Monocytes or dendritic cells function as main antigen-presenting cells. Examples of a physiologically active substance may include a cytokine and an immunoglobulin. Such a cytokine is a protein-type physiologically active substance that controls the proliferation and differentiation of various types of blood cells. Examples of such a cytokine may include interleukin (IL), a colony-stimulating factor (CSF), and chemokine. In recent years, it has been suggested that abnormal secretion of such cytokines or a failure in the control thereof are closely associated with various types of pathologic conditions. In addition, reduction in the generation of such cytokines is highly likely to result in an immunodeficient state in a severe infectious disease. Moreover, such an immunoglobulin (Ig) is a protein having functions and a structure as an antibody. Such an immunoglobulin has IgG, IgM, IgA, IgD, and IgE. IgG and IgA have their subclasses (G1 to G4, and A1 and A2, respectively). These subclasses are also included in the aforementioned immunoglobulin of the present invention.

[0028]

B cells are lymphocytes, which express an Ig receptor on the surface or inside thereof. B cells generate immunoglobulins such as IgG, IgM, IgA, or IgD, or cytokines such as IL-6. T cells are lymphocytes, which are associated with immune response and differentiate and maturate in thymus gland. Such T cells generate IL-2 to IL-6, IL-9, IL-10, IL-13, IL-14, IL-16, or the like. Dendritic cells are cells having dendrites, which act as auxiliary cells

(accessory cells) when immune response initiates. Such dendritic cells express a class II major histocompatibility (MHC) antigen, and thus function as antigen-presenting cells to helper T cells. NK (natural killer) cells exhibit cytotoxicity to virus-infected cells or tumor cells without restriction by the MHC antigen. NKT (natural killer T) cells are cells having a T cell receptor and an NK cell marker (for example, CD16 or CD56). Such NKT cells generate IFN-γ or IL-4 as a result of stimulation by α galactosylceramide (αGalCer) that is a glycolipid.

3. Confirmation of chimerism and generation of antibody

Expression of human-derived cells in a recipient animal can be confirmed by collecting peripheral blood, bone marrow cells, or other immune tissues from the recipient animal, and then confirming that the thus collected items are derived from the human.

[0029]

For example, when an immunodeficient mouse is used as a recipient, peripheral blood is collected from the retroorbital plexus thereof, or bone marrow cells are collected from femur and tibia thereof, 3 weeks to 3 months after the transplantation. In addition, spleen, lymph node, and thymus gland thereof are excised and then fragmented. Thereafter, the separated cells are passed through a mesh filter, so as to obtain a single cell suspension. These cells are then subjected to the expression analysis of human CD45 (a leukocyte common antigen, that is, a main membrane glycoprotein of hematopoietic cells), using FACSCalibur or FACSVantage (Becton Dickinson). As a result, it is identified that the cells are hematopoietic cells derived from the donor. It is also possible to stain the cells with a mouse anti-human antibody or the like.

[0030]

Moreover, in the mammal of the present invention, an immune system derived from a human as a donor has been established. Accordingly, B cells (antibody-generating cells) that are immunocompetent cells, spleen cells containing such B cells at a high rate, or other cells are stimulated with an antigen or a suitable stimulator, so as to generate a human-derived antibody. Since the surface antigen of B cells exhibits CD19 positive ($CD19^+$), the ability to generate an antibody is measured by analyzing with a cell sorter the expression of IgM, IgG, IgD, and IgA in the $CD19^+$ cells.

[0031]

Furthermore, the mammal of the present invention is immunized with a certain antigen or a suitable stimulator, and an antibody is then collected from the obtained immunocytes, so as to obtain a donor- (human-) derived antigen-specific antibody.

[0032]

In the case of mice, the amount of such an antigen or a suitable stimulator administered per animal is between 10 μg and 1 mg. Such amount is adjusted, as appropriate, depending on the presence or absence of an adjuvant. Examples of such an adjuvant may include Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), and aluminum hydroxide.

[0033]

The type of an antigen or a suitable stimulator is not particularly limited. Examples may include a protein, a peptide, and a lectin.

[0034]

The site of administration is vein, subcutis, footpad (food pad), or abdominal cavity. Immunization interval is not particularly limited. Immunization is carried out 1 to 3 times at intervals of several days to several weeks, and preferably at intervals of 1 to 2 weeks. Thereafter, approximately 1 to 2 weeks after the final immunization, an antibody titer in serum or blood plasma is measured, and antiserum or antiplasma is obtained. Such an antibody titer can be measured by enzyme-linked immunosorbent assay (ELISA), radioimmuno assay (RIA), or the like.

[0035]

When purification of an antibody from such antiserum or antiplasma is necessary, known methods such as the ammonium sulfate salting-out method, ion exchange chromatography, gel filtration, or affinity chromatography, are appropriately selected, or these methods are used in combination, so as to purify an antibody.

4. Tests regarding other chimerisms

(1) Histological analysis

A recipient mouse is dissected. Thereafter, tissues are immobilized or frozen. Tissues immobilized with paraformaldehyde are preferably dehydrated with stepwise concentrations of alcohol and are then embedded in paraffin. Sections are prepared using a microtome, a cryostat, or the like. Each of the sections is then subjected to common immunohistological staining.

[0036]

(2) Fluorescent *in situ* hybridization (FISH) method

The FISH method is a known technique of determining gene locus on chromosome. This method comprises allowing single-stranded probe DNA labeled with a fluorescent

substance or the like to hybridize with chromosomal DNA at a complementary site thereof, and then identifying a specific site in an object cell or the like under a microscope.

[0037]

[EXAMPLES]

The present invention will be more specifically described in the following examples. However, these examples are not intended to limit the present invention.

[0038]

[Example 1] Transplantation of human hematopoietic cells into immunodeficient mice

Cord blood (CB) cells were obtained from the Metro Tokyo Red Cross Cord Blood Bank. After obtaining written informed consent, CB cells were collected from cord blood to be wasted. Thereafter, monocytes (MNCs) were isolated from the CB in a density gradient manner (lymphocyte isolation medium, ICN Biomedicals) by centrifugation at 370 x g for 30 minutes. The obtained MNCs were then incubated together with mouse anti-human CD3, CD4, and CD8 antibodies (BD Immunocytometry) at 4°C for 30 minutes. After washing, the cells were incubated together with sheep anti-mouse immunomagnetic beads (DINAL) at 4°C for 30 minutes, so as to recover unbound cells. In order to isolate a $CD34^+$ population, T cell-eliminated sample was incubated together with anti-human CD34 microbeads (Miltenyi Biotech) for 40 minutes (in accordance with protocols provided by manufacturers). The cells were passed through a magnetic column twice. As a result, the purity of the concentrated $CD34^+$ cells was found to be 90% or more.

[0039]

The thus prepared CB cells ($CD3^-CD4^-CD8^-CD34^+$; 1 x 10^5 cells) were transplanted by intravenous injection into newborn NOD/SCID/B2M mice (Jackson Laboratory), the entire bodies of which had previously been irradiated with 100 cGy, so as to produce mice, in which a human immune system including human-derived immunocytes was constructed.

[Example 2] Analysis of human-derived B lineage cells in living bodies of mice

In order to examine the presence or absence of reconstruction of human lymph cells in the living bodies of mice, multiple analyses of hematopoietic tissues were conducted in terms of the transplantation level of $CD19^+$ cells (B cells) that are human $CD45^+$ cells.

[0040]

After transplantation, recipient mice were bred for 3 months, and on the 3rd month, it was analyzed regarding whether or not human-derived B lineage cells existed in the bone marrow (BM), spleen, peripheral blood (PB), and lymph nodes (LN) of the recipient mice.

The BM, spleen, PB, and LN were stained with FITC-binding immunoglobulin and PE-binding CD19.

[0041]

The results are shown in Figures 1. In Figure 1, a, b, c, and d represents the flow cytometry of cells collected from the BM; the spleen; the PB; and the LN, respectively. In each of lymphoid tissues, human $CD45^{+}CD19^{+}$ cells were identified at a high level. Each of the numerical values shown in a to d of Figure 1 (64.6, 23.6, 50.1, and 44.6, respectively) represents the ratio (%) of the $CD45^{+}CD19^{+}$ cells to the entire cells collected from each tissue.

[0042]

In addition, in 16 panels shown in Figure 1, panels in the first column (e), panels in the second column (f), panels in the third column (g), and panels in the fourth column (h) represent the results obtained by staining the hematopoietic cells derived from the BM, the spleen, the PB, and the LN with FITC-binding IgM (at the first line), IgD (at the second line), IgG (at the third line) and IgA (at the fourth line), and PE-binding CD19 antibody, respectively. The numerical value in each panel represents the ratio (%) of cells expressing each class of immunoglobulin in the $CD19^{+}$ cells. For example, the numerical value, 90.1, found in the line of (e) and in the first column represents the ratio of bone marrow-derived cells expressing IgM.

[0043]

From these results, it was revealed that human-derived immunoglobulins are expressed at a high rate in the tissues of each of BM, spleen, PB, and LN.

[0044]

Subsequently, in order to examine the antigen-specific response of human lymphoid cells surviving in mice, the mice produced in Example 1 were immunized with 100 μg of ovalbumin (OVA), and the presence of OVA-specific IgM and IgG was then analyzed by ELISA. The blood plasma of recipient mice was diluted 10 times (for IgM analysis) or 3 times (for IgG analysis), and the absorbance of each sample was then measured. At the same time, B cells were collected from the recipient mice, and the cells were then cultured in RPMI/FCS (fetal bovine serum)/Pokeweed mitogen medium for 5 days. Thereafter, the immunoglobulin contained in the culture supernatant was measured by ELISA. As a negative control, the immunoglobulin contained in human serum was measured.

[0045]

The results are shown in Figure 2. In Figure 2, panel a represents the results of IgM, and panel b represents the results of IgG. The bars in the figure represent blood plasma, culture supernatant, negative control 1 (human serum), and negative control 2 (a tenth part of

the serum as negative control 1), from the left. Figure 2 shows that antigen-specific IgM and IgG were generated at a high rate.

[Example 3] Differentiation and maturation of B lineage cells

Using a cell sorter, human $CD19^+$ cells (B cells) were collected from the peripheral blood (PB), bone marrow (BM), and spleen of recipient mice, and the expression of IgM, IgG, IgD, and IgA in the B cells was examined. The surface expression of IgM/IgD in the $CD19^+$ cells was 90.0%/54.0% in the case of PB, 19.7%/3.4% in the case of BM, and 59.0%/22.7% in the case of spleen.

[0046]

Using pokeweed mitogen (PWM), the spleen cells were further cultured in a test tube for 5 days. In addition, recipient mice immunized with 100 μg/ml of OVA were also produced. Subsequently, secretion of human immunoglobulins into the culture supernatant and blood plasma was examined by ELISA (Table 1). After completion of the culture with PWM for 5 days, the medium (supernatant) contained 114 ng/ml to 19.8 μg/ml of IgM, 2.6 to 47.6 ng/ml of IgG, and 1.9 to 5.7 ng/ml of IgA (Table 1).

[0047]

Table 1 Generation of human immunoglobulins

Sample	Plasma/immunogen	IgM	IgG	IgA
1	Plasma/OVA	225000	823	553
2	Medium/OVA	19800	47.6	5.7
3	Plasma	23000	13	40
4	Medium	114	2.6	1.9
5	Plasma	47700	4.1	10.3
6	Plasma	17200	5	9.4

(Unit of amount generated: ng/ml)

[0048]

Blood plasma colleted from the recipient mice contained 17.2 to 225 μg/ml of IgM, 4.1 to 823 ng/ml of IgG, and 9.4 to 553 ng/ml of IgA.

[0049]

As shown in Table 1, when recipient mice were immunized with OVA, human B cells secreted large amounts of IgM, IgG, and IgA, including OVA-specific IgM. Accordingly, in the present example, it was shown that human B cells generated in newborn NOD/SCID/B2M mice maturate and then generate human-derived IgM and IgD, and that the above cells have

functions to generate antigen-specific human-derived IgM, IgG, and IgA.

[0050]

Based on these findings, it was revealed that B cells obtained from the mouse of the present invention are not only used as human antibody-generating cells, but also useful for generating human immunoglobulins (monoclonal antibodies) acting on pathogenic microorganisms causing severe infectious diseases or on tumors.

[Example 4] Analysis of human-derived T lineage cells in living bodies of mice

In the present example, flow cytometric analysis was carried out regarding the presence of human T cells (CD45 and CD3) in the BM, spleen, and PB of recipient mice.

[0051]

The results are shown in Figure 3. The panels in Figures 3a to 3c represent the analysis results of BM (a), those of spleen (b), and those of PB (c), respectively. When compared with B cells, the number of T cells was smaller, but the T cells differentiated. Three months after the transplantation into the recipient mice, the ratio of $CD3^+$ cells was found to be 0.17% in the case of BM, 1.44% in the case of spleen, and 1.8% in the case of PB.

[0052]

The ratio of antigen-presenting cells (APCs) with phenotype $HLA^-DR^+CD11c^+$ was found to be 1.09% in the case of BM (Figure 3d). Moreover, in thymus gland, $CD19^+IgM^+$ B cells were identified (Figure 3e).

[Example 5] FISH analysis and immunofluorescence analysis of lymphoid tissues

In order to examine distribution of human lymph cells *in situ*, using spleen derived from the recipient mice, double FISH analysis was carried out in terms of human and mouse chromosomes. FISH was carried out in accordance with a common method.

[0053]

From an experiment using human X chromosome probe, human cells were obtained at a high frequency, which did not contradict the results of FACS analysis. From the results of double FISH analysis using human and mouse X chromosomes, it became clear that interstitial cells derived from mice also exist in the spleen (Figure 4).

Human cells were identified as green signals (human X chromosomes) (Figure 4a). Several spleen cells derived from the recipient mice were stained with mouse anti-human CD3, and such cells became red (Figure 4c). Figure 4d is a view obtained by overlaying panel a on panel b. The blue-stained portion represents a nucleus.

[0054]

Before transplantation, recipient mice lacked all mature lymph cells. By transplanting human CB-derived T cell-eliminated $CD34^+$ cells into the mice, human-derived lymphoid tissues were successfully reconstructed in the mice.

[0055]

In addition, tissue samples were subjected to immunohistological staining. The results obtained by immunohistological staining of spleen tissues collected from mice are shown in Figures 4d and 4e. A majority of spleen cells were stained with anti-human IgM positive (d) and anti-human IgD positive (e), and they became red. Thus, it was revealed that human-derived spleen cells survived therein at an extremely high rate. Moreover, the spleen tissues were stained with mouse anti-human CD3. As a result, a portion of the spleen was stained with anti-human CD3, and it became positive (red) (Figure 4f). Thereafter, a specific antibody to follicular dendritic cells was used to conduct immunostaining, and the presence of human APCs was also confirmed (Figure 4g).

[0056]

[Effect of the invention]

The present invention may provide a method for producing human-derived immunocompetent cells using a newborn immunodeficient animal. Since the newborn immunodeficient animal of the present invention is able to construct a human-derived immune system in the body thereof, it is useful for the functional analysis of lymphoid tissues and the production of a human-derived antibody using B cells.

[Brief description of the drawings]

[Figure 1] Figure 1 shows the reconstruction of a human B cell line ($CD19^+$ cells) in recipient mice.

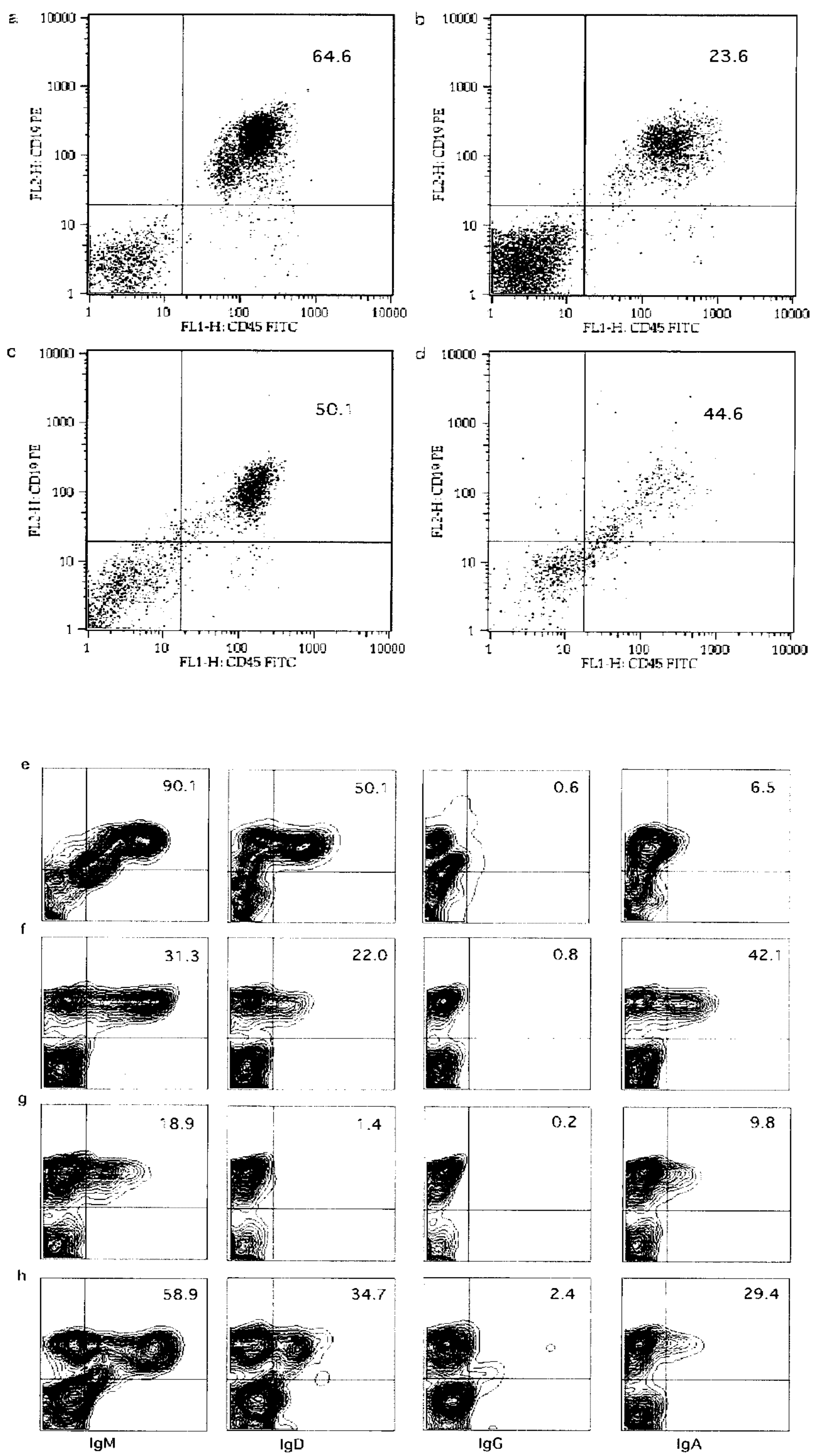
[Figure 2] Figure 2 shows the results of ELISA performed on OVA-specific IgM.

[Figure 3] Figure 3 shows the reconstruction of a human T cell line in the bone marrow, spleen, and peripheral blood of recipient mice.

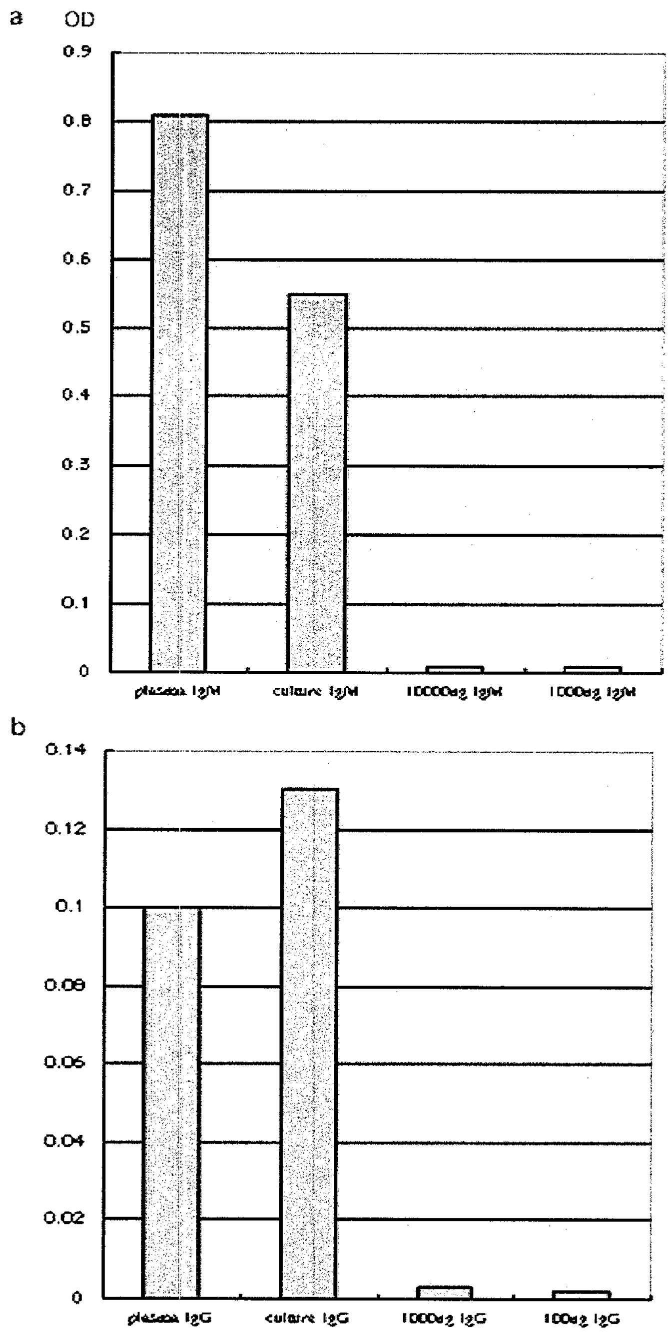
[Figure 4] Figure 4 shows the results obtained by the FISH analysis and immunohistological analysis of lymphoid tissues.

[Name of Document] DRAWINGS

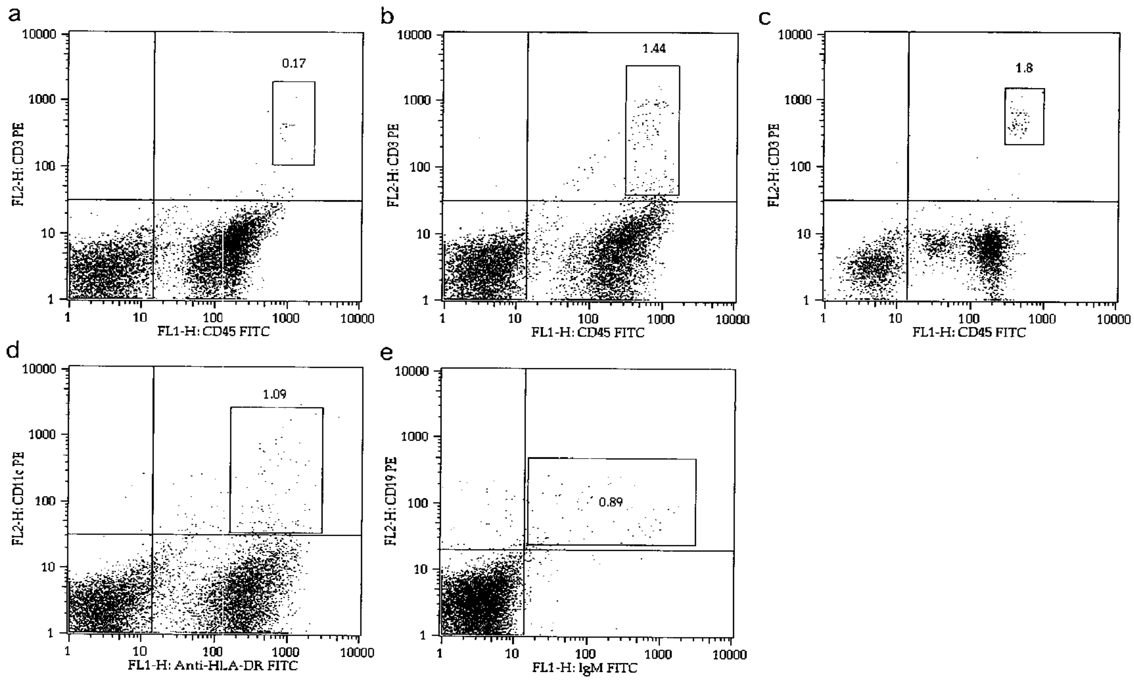
[Figure 1]



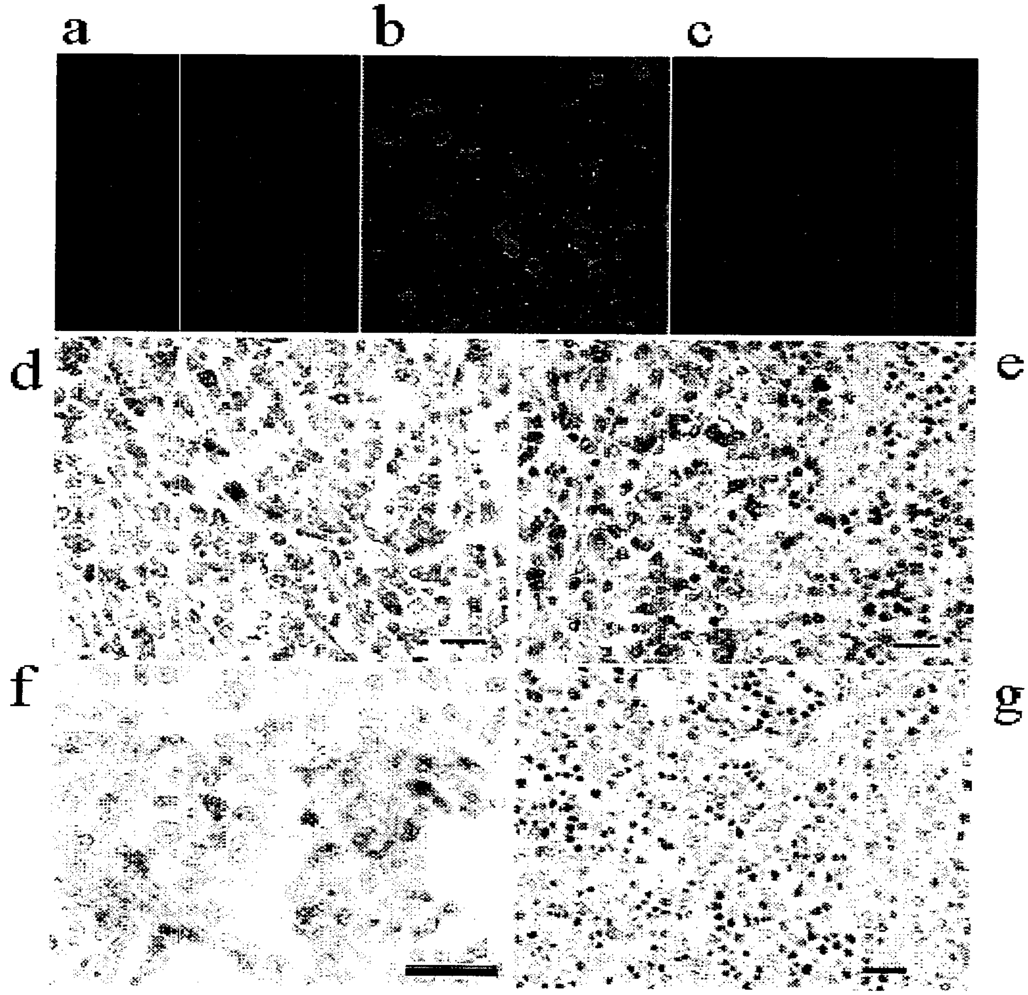
[Figure 2]


a
OD
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
plasma IgM
culture IgM
10000xg IgM
1000xg IgM
b
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
plasma IgG
culture IgG
1000xg IgG
100xg IgG

[Figure 3]


a
10000
1000
100
10
1
FL2-H: CD3 PE
0.17
1
10
100
1000
10000
FL1-H: CD45 FITC
b
1.44
FL1-H: CD45 FITC
c
1.8
FL1-H: CD45 FITC
d
FL2-H: CD11c PE
1.09
FL1-H: Anti-HLA-DR FITC
e
FL2-H: CD19 PE
0.89
FL1-H: IgM FITC

[Figure 4]



[Name of Document] ABSTRACT

[Abstract]

[Problem] To provide an immunodeficient animal capable of generating human-derived lymphoid cells, a human-derived lymphoid cell, and a method for producing a human antigen-specific antibody.

[Means for solving the problems] A newborn immunodeficient mammal into which human-derived hematopoietic precursor cells have been transplanted, and which is able to generate said human-derived hematopoietic cells or immunocompetent cells; and a method for producing a human-derived antibody, which is characterized in that it comprises recovering immunocompetent cells from the above-described mammal, culturing the immunocompetent cells, and collecting a human-derived antibody from the obtained culture product.

[Representative drawing] None